REP 1996 -107

EXPOSURE TO VOLATILE D-PULEGONE ALTERS FEEDING BEHAVIOR IN EUROPEAN STARLINGS

S. A. WAGER-PAGÉ, U. S. Department of Agriculture, Animal and Plant Health Inspection Service, Animal Damage Control, Denver Wildlife Research Center, c/o Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, USA
J. R. MASON, U. S. Department of Agriculture, Animal and Plant Health Inspection Service, Animal Damage Control, Denver Wildlife Research Center, Utah State University, Logan, UT 84322, USA

Abstract: D-pulegone repels birds, but it is unclear if its volatile cues can stimulate avoidance behavior or whether direct contact is required for this response. We used European starlings (*Sturnis vulgaris*) in feeding tests to investigate the role of volatile cues in d-pulegone's repellency. Direct contact with d-pulegone (1.0% vol/vol) coating decreased apple consumption by 53% ($P < 0.02$). When direct contact was prevented by encapsulating d-pulegone (10.0% vol/vol) in HistoPrep® capsules (Fisher Scientific, Pittsburgh, Pa.) or applying the stimulus solution to the underside of the feeding cup lids, ingestion of test foods was inhibited. The inhibitory effect of the volatile cues of 100% d-pulegone on feed ingestion was increased following repeated exposures. These results show that d-pulegone volatiles induced avoidance behavior in European starlings although direct contact with the compound produced a stronger response.

J. WILDL. MANAGE. 60(4):917–922

Key words: d-pulegone, European starlings, feeding behavior, frugivory, *Mentha* sp., *Sturnis vulgaris*, volatile repellent.

Birds depredate agricultural commodities resulting in significant economic loss (Mason and Clark 1992:115–129). The few chemical repellents that could resolve these problems are limited in the types of situations where they can be applied (Thomson 1995). Frugivorous birds, including European starlings cause millions of dollars in damage to fruit crops in the United States. Since the registration of methiocarb (3,5-dimethyl-4-[methylthio]phenol methyl carbamate) expired in 1987, there are no available chemical repellents registered by the Environmental Protection Agency for application to fruit crops. Pen and field trials evaluating potentially useful nonlethal repellents such as methyl anthranilate formulations and Keyplex-350 demonstrated that these compounds have limited efficacy when applied to fruit crops (Curtis et al. 1994, Cummings et al. 1995).

Most deterrent compounds require physical contact to be effective, although, ideally, a repellent should be active via a volatile cue to prevent sampling. Promising sources of natural repellent substances are volatile compounds produced by plants as a defense against herbivory. For example, terpenoid compounds reduce feeding by a number of avian and mammalian species including snowshoe hares (*Lepus americanus*) (Bell and Harestad 1987), voles (*Microtus* sp.) (Bell and Harestad 1987, Roy and Bergeron 1990), pocket gophers (*Thomomys* sp.) (Radwan et al.1982), and brown-headed cowbirds (*Molothrus ater*) (Mason and Bonwell 1993). Coniferyl and cinnamyl derivatives, structurally related phenylpropanoids, inhibit feed intake of European starlings during laboratory trials (Jakubas et al. 1992).

D-pulegone, a monoterpene present in pennyroyal (*Mentha pulegium*) (Duke 1987), is an effective avian repellent that reduces feeding in European starlings (Mason 1990). Ingestion of pesticides that are mistaken for grit because of their visual and textural similarities, results in the accidental poisoning of many birds. Treatment of clay particles with d-pulegone decreased ingestion of granules by northern bobwhites (*Colinus virginianus*) (Mastrota and Mench 1995). Whether volatile d-pulegone will effectively repel birds before ingestion or whether direct contact with the substance is required is unknown. Ingestion of even small quantities of some granular pesticides may be fatal to birds (i.e. aldicarb, diazinon, and fensulfothion) (Balcomb et al. 1984, Hill and Camardese 1984). Airborne repellents that are effective before birds contact pesticide granules could decrease poisonings. The repellency of d-pulegone in feeding trials was evaluated to determine its effectiveness when birds were exposed only to its volatile cues.

We examined the effectiveness of d-pulegone as a feeding deterrent when applied as a coating solution to protect a fruit product, apple, from depredation. Typically, a repellent compound is applied to the surface of blueberries, grapes, and cherries by spraying (Curtis et al. 1994, Cummings et al. 1995, Dolbeer et al. 1994). Birds feed on fruit either by ingesting small fruits whole when their gape width is adequate or by pecking and swallowing the pulp. Contact with the repellent could be minimized as the birds peck through the surface gaining access to the unadulterated pulp. We evaluated d-pulegone's repellency following application as a coating to apple.

Although apples and other fruits are readily consumed by European starling, these foods are available seasonally and would constitute only a portion of the birds' diets in the wild. Avoidance of apples, a dietary supplement for the captive birds, would not have a major effect on their nutritional status. D-pulegone's effect on the consumption of feed was evaluated to determine whether this compound can decrease intake of the regular laboratory ration of these captive birds.

We thank S. Lundy and A. Ottinger for technical assistance. This work was supported by a cooperative agreement 12-31-41-0040(CA) between the Denver Wildlife Research Center and the Monell Chemical Senses Center.

METHODS

Thirty-six adult European starlings of unknown sex were randomly selected from the laboratory colony for use in these studies. All birds were individually caged (61 × 36 × 41 cm) under a 12:12 light:dark cycle at 23 C for the duration of the study. Before the experiment, the birds were allowed free access to Purina Flight Bird Conditioner® (Purina, St. Louis, Mo.) and crushed shell grit (collectively referred to below as feed), and tapwater. All birds were used in 2 studies with 2–4 week rest periods between tests except for the birds that were used in the habituation studies. The rest period in the habituation study was 1 week. Starlings were exposed to the d-pulegone concentrations in ascending order.

D-pulegone (CAS#89-82-7) was donated by International Flavors and Fragrances (Union Beach, N.J.). D-pulegone was dissolved in vegetable oil purchased at WaWa Food Market (Philadelphia, Pa.) to produce coating solutions with the following concentrations: 0.01, 0.1, 1.0 and 10% vol/vol.

Experiment 1

We evaluated the effect of d-pulegone (0.01, 0.1 and 1.0 % vol/vol) coating on apple ingestion by European starlings. Two groups of starlings (n = 12) were randomly selected from the laboratory colony. At the start of each experiment, birds were adapted to an overnight food deprivation regime (1700–0900 hr) and familiarized with eating apples. Testing was performed on 4 consecutive days with 2 days of pretest baseline intake followed by 2 days of exposure to the d-pulegone and control stimuli in 2 hour, 2 choice tests. Within 1 hour of lights on (0800 hr), all birds were presented with 2 apple quarters placed in the opposite front corners of their home cages. During the 2 day pretest period, both apple quarters were coated with the vegetable oil only. On treatment days, each bird was presented with 2 apple quarters, 1 coated with d-pulegone solution and the other with vegetable oil. Each test was performed in duplicate and the positions of the treatment and control stimuli presentations were counterbalanced. After 2 hours, apple quarters were removed and weighed. From 1100–1700 hours, all birds had free access to feed and tapwater.

Experiment 2

We evaluated the effect of volatile d-pulegone on apple ingestion by European starlings. The experimental procedure was similar to that described above except the birds were presented with preweighed apple quarters paired with either d-pulegone (10% vol/vol) or oil saturated filter paper discs encased in plastic mesh HistoPrep® (Fisher Sci., Pittsburg, Pa.) tissue capsules (38 × 8 mm) during 2-hour, 2-choice tests which prevented direct contact with the stimulus solution. Apple quarters were attached to the capsules with plastic ties and placed in the opposite corners of the birds' home cages. Pretest baseline intake data were collected on 2 consecutive days preceding the test period in 2-hour, 2-choice tests. During the pretest period, oil saturated filter paper discs were encased in both HistoPrep® capsules and attached to apples. At the completion of each 2-hour test period, apple quarters and HistoPrep® capsules were removed and reweighed to measure intake. All tests were performed in duplicate and the position of apples + HistoPrep® capsules containing d-pulegone or oil were counterbalanced to control for side preferences.

Experiment 3

We evaluated the effect of volatile d-pulegone (10% vol/vol) on ingestion of feed by European starlings during 2-hour, 2-cup tests. Food cups were removed at 1600 hours on the afternoon preceding each test day and returned at the end of the trial. Feed (150 g) was placed in aluminum feeding cups (diam: 80 mm, ht: 40 mm) and covered with stainless steel lids having a 32-mm opening in the center. To prevent the birds from directly contacting the stimuli, 100 μl or d-pulegone (10% vol/vol) was applied to the underside of the control and treatment cup lids, respectively. The preweighed food cups were placed in opposite corners of the birds' home cages at 0900 hours, removed at 1100 hours, and weighed to determine feed consumption. Pretest baseline intake data were collected as previously described, except feed consumption was measured rather than apple ingestion and oil was applied to the underside of both cup lids. All tests were performed in duplicate and the position of cups treated with d-pulegone and oil was counterbalanced to control for side preferences.

Experiment 4

We evaluated the effect of multiple exposures to volatile d-pulegone (100% vol/vol) on feed intake and body weight of European starlings. The procedures followed were the same as described in experiment 3 except European starlings (n = 12) were exposed to volatile d-pulegone on 6 consecutive days. Pretest baseline intake data were collected on 2 days preceding the test period. Body weights of the birds were obtained at the end of the pretest period immediately preceding test day 1 and again on test day 6 and post-test day 5 to monitor variations in body weight during the experimental period. The average intake from the oil and d-pulegone treated feed cups presented on the left and right sides was calculated for each test period. Post-test intake was measured on 2 days following the 6-day test period. Birds were weighed before and after the test period.

Analysis

For each experiment, we used an ANOVA to evaluate food intake. A statistical software package, (Clear Lake Res. Inc. 1986) was used to analyze the data. Differences were considered significant if $P < 0.05$ was achieved in Tukey's post-hoc tests.

European starlings exhibit marked side preferences; therefore, all 2-choice tests were performed in duplicate with the presentation order of the treatment and control stimuli counterbalanced. Data collected from the 2 sides were combined and averaged for all tests except for the data from experiment 4 that was used to evaluate the effect of d-pulegone on side preferences.

In experiment 1, apple intakes were compared by a 2-factor ANOVA with d-pulegone concentration (3 levels: 0.01–1.0% vol/vol) as the between-subject factor and choice (2 levels: vegetable oil coating, d-pulegone coating) as the within-subject factor.

In experiments 2 and 3, apple and feed intakes following exposure to d-pulegone's (10% vol/vol) volatile cues were compared to ingestion following exposure to the control stimuli using 1-factor ANOVAs Preference ratios were calculated to eliminate the effect of different baseline intakes when comparing the effect of d-pulegone on feed and apple ingestion Preference ratio = apple or feed associated with d-pulegone volatiles/(amt of apple or feed associated with d-pulegone volatiles eaten + amt of apple or feed associated with vegetable oil eaten) × 100%.

In experiment 4, feed intakes were compared using a 2-factor ANOVA with test period (3 levels: period 1–3) as the between-subject factor and choice (2 levels: vegetable oil, d-pulegone) as the within-subject factor. Total feed intakes during the pretest and test periods were compared by a 1-way ANOVA with periods as the repeated measure. In experiment 4, a 2-factor ANOVA was used to evaluate the effect of d-pulegone on side preferences and the between factors were side (2 levels: left, right) and choice (2 levels: vegetable oil, d-pulegone).

RESULTS

In the first experiment, 1.0% d-pulegone coating inhibited apple consumption by European starlings,($F = 6.0203$, 1,32 df, $P < 0.02$) (Fig. 1). Total 2-hour combined intake of vegetable oil and d-pulegone coated apple pieces during the test periods did not differ from total con-

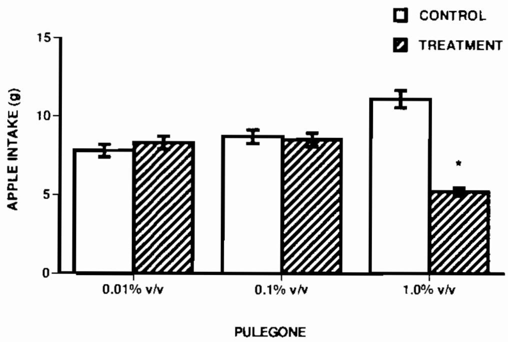

Fig. 1. Effect of d-pulegone (0.01–1.0% vol/vol in oil) on 2-hour apple consumption by European starlings in 2-choice tests. Data are expressed as mean amounts of apple ingested in grams (g). (*) $P < 0.05$. Capped vertical bars represent standard errors of the means.

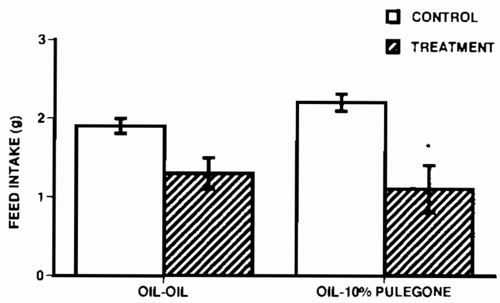

Fig. 3. Effect of the volatile cues of d-pulegone (10.0% vol/vol) on feed ingestion by European starlings in 2-choice tests compared to the control stimuli, oil, on control (oil-oil) and treatment days (oil-10% d-pulegone). Data are expressed as mean amounts of feed ingested in grams (g). (*) $P < 0.05$. Capped vertical bars represent standard errors of the means.

sumption of unadulterated apples during the pretest periods.

When direct contact with the stimulus solutions was prevented in the second experiment, exposure to d-pulegone's volatile cues decreased apple ingestion compared to exposure to vegetable oil, ($F = 5.034$, 1,20 df, $P < 0.036$) (Fig. 2). When d-pulegone (10% vol/vol) was applied to the underside of cup lids, intake of feed from these cups was inhibited compared to ingestion from the cups with oil treated lids, ($F = 5.387$, 1,20 df, $P < 0.03$) (Fig. 3). Evaluation of the preference ratios comparing the effects of volatile d-pulegone on apple and feed ingestion indicated that intake of both foods was inhibited to a similar extent (apple intake: $39.3 \pm 4.7\%$, feed intake: $40.6 \pm 4.7\%$) ($F = 0.016$, 1,20 df, $P > 0.9$). The total combined 2-hour intakes of feed or apples associated with vegetable oil and d-pulegone during the test periods did not differ from total consumption of unadulterated test foods during the pretest periods in experiments 2 and 3 (data not shown).

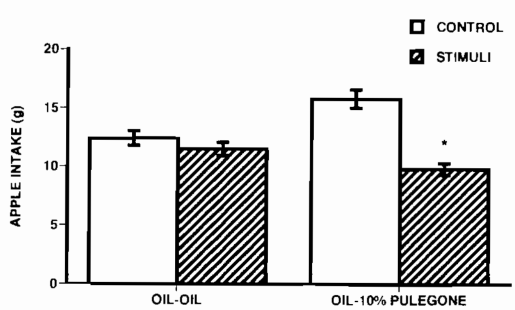

Fig. 2. Effect of the volatile cues of d-pulegone (10.0% vol/vol) on apple ingestion by European starlings in 2-choice tests compared to the control stimuli, oil, on control (oil-oil) and treatment days (oil-10% d-pulegone). Data are expressed as mean amounts of apple ingested in grams (g). (*) $P < 0.05$. Capped vertical bars represent standard errors of the means.

In the final experiment there was an interaction between concentration and period, with the effect of d-pulegone on total 2-hour feed consumption increasing over the 3 periods resulting in a lower 2-hour intake during the test periods than the pretest period, ($F = 4.62$, 2,44 df, $P < 0.015$) (Fig. 4). Association with d-pulegone's volatile cues decreased feed intake compared to ingestion from cups associated with vegetable oil cues during test periods 1 and 2 ($F = 11.076$, 1,33 df, $P < 0.002$); however, this effect was not apparent during test 3. During test 1, starlings exhibited a preference for feeding from the left side, ($F = 11.564$, 1,44 df, $P < 0.001$) (Fig. 5). Following repeated exposures to volatile d-pulegone, European starlings failed to maintain their left side preference and selected equivalent amounts of feed from cups with oil and d-pulegone treated lids in either position (Fig. 5).

Body weights of 8 of the 12 birds exposed to 100% d-pulegone decreased by the end of test 3. At the beginning of the pretest period, mean body weight was 80.7 ± 2.4 g compared to 77.0 $\pm$ 1.9 g at the end of the treatment period, ($F = 8.96$, 2,22 df, $P < 0.001$). Most, but not all, of the European starlings that lost weight during the d-pulegone test periods began to replace this deficit by 5 days post-test.

DISCUSSION

Contact with d-pulegone coating inhibited ingestion of apples by European starlings. Our results agree with findings from earlier studies

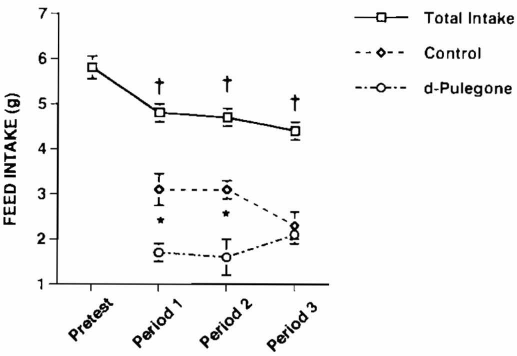

Fig. 4. Effect of repeated exposure to the volatile cues of d-pulegone on 2-hour feed consumption by European starlings in 2-choice tests during the pretest period and 3 2-day test periods. Data are expressed as mean amounts of feed ingested in grams (g) from cups with either the control (oil) and d-pulegone coated lids and the total 2-hour consumption from both cups. (*) $P < 0.05$ indicates differences in ingestion from control and treatment cups in each test period. (†) $P < 0.05$ indicates differences in total 2-hour consumption across test period compared to the pretest period. Capped vertical bars represent standard errors of the means.

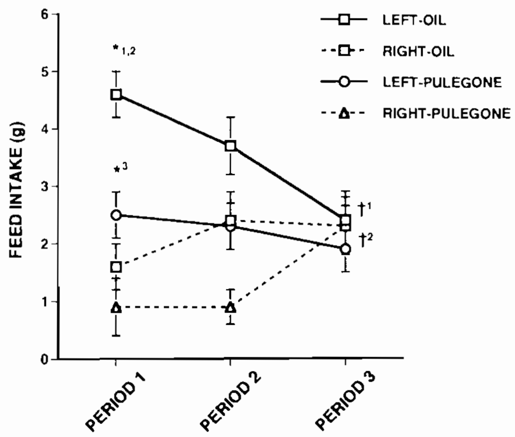

Fig. 5. Effect of repeated exposure to the volatile cues of d-pulegone on side preference by European starlings in 2-choice tests. Data are expressed as mean amounts of feed ingested in grams (g) from cups in the left and right sides when associated with the oil (left-oil, right-oil) or d-pulegone (left-pulegone, right-pulegone) coated lids. (*) $P < 0.05$ differences in intake between left and right sides when associated with either oil or d-pulegone within individual test period ([*1,2] compared to left-pulegone and right-oil, [*3] compared to right-pulegone). (†) $P < 0.05$ differences in intake across periods, ([†1] left-oil period 3 compared to left-oil period 1, [†2] right-pulegone period 3 compared to period 1). Capped vertical bars represent standard errors of the means.

that d-pulegone deterred ingestion of feed and granular particles (Mason 1990, Mastrota and Mench 1995). In previous studies, intake was decreased when feed was adulterated with d-pulegone concentrations as low as 0.01% (wt/wt) (Mason 1990). In our first experiment, we chose to apply coating solutions directly to the surface of apples providing a homogeneous repellent barrier rather than incorporating the compound into the test food. Comparisons of our findings with those obtained from earlier studies are difficult because exposure to d-pulegone incorporated into test foods and granules differs greatly from contact with a surface coating.

Volatile d-pulegone decreased ingestion of apples as well as the regular laboratory feed diet by European starlings. These findings demonstrate that d-pulegone will effectively deter consumption of food in the absence of direct contact. In experiments 2 and 3, starlings maintained their total 2-hour consumption at levels equivalent to those observed during the pretest period. Compensation was achieved by increasing ingestion of the alternative food source during 2-choice tests. Therefore, in an environment with alternative food sources present, the volatile cues of d-pulegone are sufficient to deter ingestion of the protected food.

European starlings decreased their total 2-hour feed consumption following repeated exposures to volatile d-pulegone (100% vol/vol). An interaction between d-pulegone versus oil control and test period was observed with the inhibitory effect of d-pulegone increasing over time. The data suggest that in the presence of a high concentration of volatile d-pulegone, feeding was decreased through a generalized aversive response.

In earlier studies birds did not lose body weight following repeated contact with d-pulegone, (Mason 1990). When we exposed birds to 100% d-pulegone for 6 days, 8 of 12 birds lost a few grams of body weight. By 5 days post-test, most of the birds had begun to replace their body weight deficit. This small weight loss may indicate a prolonged appetite suppressant effect of d-pulegone reducing their total daily food intake. Alternatively, d-pulgone may have a slight toxic effect causing malaise. Future testing will be necessary to address these issues.

MANAGEMENT IMPLICATIONS

European starlings decreased consumption of apple that had been protected by a 1.0% d-pulegone coating. These findings demonstrate that d-pulegone has potential for development as a control agent for prevention of fruit depredation by pest birds. In many situations a repellent that can deter contact with or sampling of a substance would be desirable (i.e. reduction of accidental bird poisonings by ingestion of granular pesticides). In an environment where alternative food sources of equal nutritive or sensory value are available, direct or volatile contact with d-pulegone will decrease ingestion of the food or substance with which it is associated. Our findings demonstrate that d-pulegone is an ideal compound for use when a contact repellent is contraindicated. Whether prolonged exposures to d-pulegone will have toxic effects on avian species will require additional studies. Future studies will need to evaluate the repellency of related mentha compounds to identify products that will be both non-toxic and economically feasible to use.

LITERATURE CITED

BALCOMB, R., R. STEVENS, AND C. BOWEN. 1984. Toxicity of 16 granular insecticides to wild-caught songbirds. Bull. Environ. Contam. Toxicol. 33: 302–307.

BELL, C. M., AND A. S. HARESTAD. 1987. Efficacy of pine oil as repellent to wildlife. J. Chem. Ecol. 13:1409–1417.

CLEAR LAKE RESEARCH, INC. 1986. CLR ANOVA. Version 1.1. Clear Lake Res., Inc., Houston, Tex.

CUMMINGS, J. L., M.L. AVERY, P. A. POCHOP, J. E. DAVIS, JR., D.G. DECKER, H.W. KRUPA, AND J. W. JOHNSON. 1995. Evaluation of methyl anthranilate formulation for reducing bird damage to blueberries. Crop Prot. 14:257–259.

CURTIS, P. M., I. A. MERWIN, M. P. PRITTS, AND D. V. PETERSON. 1994. Chemical repellents and plastic netting for reducing bird damage to sweet cherries, blueberries, and grapes. Hort. Sci. 29: 1151–1155.

DOLBEER, R. A., M. L. AVERY, AND M. E. TOBIN. 1994. Assessment of field hazards to birds from methiocarb applications to fruit crops. Pestic. Sci. 50:147–161.

DUKE, J. A. 1985. CRC handbook of medicinal herbs. CRC Press. Boca Raton, Fla. 667pp.

HILL, E. F., AND M. B. CAMARDESE. 1984. Toxicity of anticholinesterase insecticides to birds: technical grade versus granular formulations. Ecotoxicol. Environ. Safety 8:551–563.

JAKUBAS, W. J., P. S. SHAH, J. R. MASON, AND D. M. NORMAN. 1992. Avian repellency of coniferyl and cinnamyl derivatives. Ecol. Appl. 2:147–156.

MASON, J. R. 1990. Evaluation of d-pulegone as an avian repellent. J. Wildl. Manage. 54:130–135.

———, AND L. CLARK. 1992. Nonlethal repellents: the development of cost-effective, practical solutions to agricultural and industrial problems. Pages 115–129 *in* J. E. Borrecco and R. E. Marsh, eds. Proc. Fifteenth Vertebr. Pestic. Conf. Univ. California, Davis.

———, AND W. R. BONWELL. 1993. Evaluation of turpentine as a bird-repellent seed treatment. Crop Prot. 12:453–457.

MASTROTA, F. N., AND J. A. MENCH. 1995. Evaluation of taste repellents with northern bobwhites for deterring ingestion of granular pesticides. Environ. Toxicol. Chem. 14:631–638.

RADWAN, M. A., G. L. CROUCH, C. A. HARRINGTON, AND W. D. ELLIS. 1982. Terpenes of ponderosa pine and feeding preferences by pocket gophers. J. Chem. Ecol. 8:241–253.

ROY, J., AND J. M. BERGERON. 1990. Role of phenolics of coniferous trees as deterrents against debarking behavior of meadow voles (*Microtus pennsylvanicus*). J. Chem. Ecol. 16:801–817.

THOMSON, W. T., EDITOR. 1995. Agricultural chemicals, book III-miscellaneous agricultural chemicals: fumigants, growth regulators, seed safeners, repellents, fish toxicants, bird toxicants, pheromones, rodenticides and others. Thomson Publ. Fresno, Calif. 209pp.

Received 23 May 1995.
Accepted 10 May 1996.
Associate Editor: Fairbrother.